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Voltammetric and HPLC techniques for the determination of paroxetine hydrochloride

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The antidepressant agent paroxetine hydrochloride (POT) was studied by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and osteryoung square wave voltammetry (OSWV). A sensitive method is described for the determination of POT in its pure form and in human plasma. The linear relationship between concentration and peak current permits the quantification of POT by CV, DPV and OSWV in the concentration range of 2×10^{-5}–8×10^{-4} M. Applicability to tablets and human plasma analysis has been illustrated. Furthermore, a HPLC method with diode array detection was developed. Linearity was established between 2×10^{-7}–6×10^{-5} M for POT. The described methods were successfully employed with high degrees of precision and accuracy for the estimation of total drug content in human plasma and pharmaceutical dosage forms of POT.

1. Introduction

Therapeutic drug monitoring of tricyclic antidepressant drugs (TCAs) is often performed to enhance therapeutic efficacy and prevent serious adverse effects. The benefit of therapeutic drug monitoring with selective serotonin reuptake inhibitors (SSRIs) is still a matter of debate. Paroxetine (POT), a new SSRI antidepressant that has the highest inhibitory activity of serotonin reuptake is now used for a variety of psychiatric conditions [1]. Paroxetine's chemical structure is unrelated to the tricyclic antidepressants as well as older selective serotonin reuptake inhibitors and thus has a potential for different drug interactions. Unlike other selective serotonin reuptake inhibitors, paroxetine has a comparatively high affinity for muscaric receptors and clinically, anticholinergic side-effects are dose-related [2].

Several analytical procedures have been developed to quantify POT and its metabolites in human serum; i.e., gas chromatography-mass spectrometry [3, 4], HPLC with electrochemical [5], UV [6–11], and fluorescence detection [12–17] and micellar electrokinetic capillary chromatography [18]. These methods require derivatization and extensive sample pretreatment prior to analysis. In addition, two HPLC methods with UV detection have been reported for determination of POT in bulk and in pharmaceutical formulations [19, 20].

To the best of our knowledge, there are no reports on the electrochemical behaviour of POT. Furthermore, an official method for the determination of this drug in pharmaceutical forms and human plasma has not been yet described in any pharmacopoeia.

The present work aimed to investigate the electrochemical behaviour of the drug in Britton-Robinson (BR) buffers and phosphate buffers at a glassy carbon electrode. It also aimed to optimize an electrochemical procedure for the direct determination of the drug (without derivatisation) in pharmaceutical formulations and human plasma using CV, DPV and OSWV techniques. The data of the voltammetric techniques were compared with a reference HPLC method.

2. Investigations, results and discussion

2.1. Voltammetric methods

Analyses were performed with a glassy carbon electrode at different scan rates in various supporting electrolyte solutions; namely 0.2 M H_2SO_4, 0.5 M H_2SO_4, phosphate buffers of pH = 3.20–10.85 and Britton-Robinson buffers of pH = 1.83, 2.02, 3.17, 4.00, 5.00, 6.01, 7.24, 8.16, and 9.22.

Fig. 1 displays a cyclic voltammogram of 10^{-4} M POT obtained in 0.5 M H_2SO_4 (a) and Britton-Robinson buffers of pH 1.83–9.22 (b–j) with a scan rate of 100 mV s^{-1}. In all curves a well defined anodic peak and a second broad one were observed. The second peak can be scaned up to pH = 5.0 and nearly disappeared at the higher pH values. The peak potentials of the first and second peaks in 0.2 and 0.5 M H_2SO_4 and Britton-Robinson solution of pH = 1.83 are about 0.95 V and 1.15 V, respectively. Peak potential pH plot showed two linear sections $Ep = -8.31\ pH + 955.6$ and $Ep = -22.33\ pH + 1059.2$, indicating that hydrogen ions take part in the reaction mechanism. Nevertheless, the mechanism is complicated. On the cathodic branch in 0.2 and 0.5 M H_2SO_4 solutions two small peaks at about 0.9 V and 0.45 V were observed (only the curve related to 0.5 M H_2SO_4 was given in Fig. 1a), but in Britton-Robinson buffers only one reduction peak at about 0.9 V appeared.

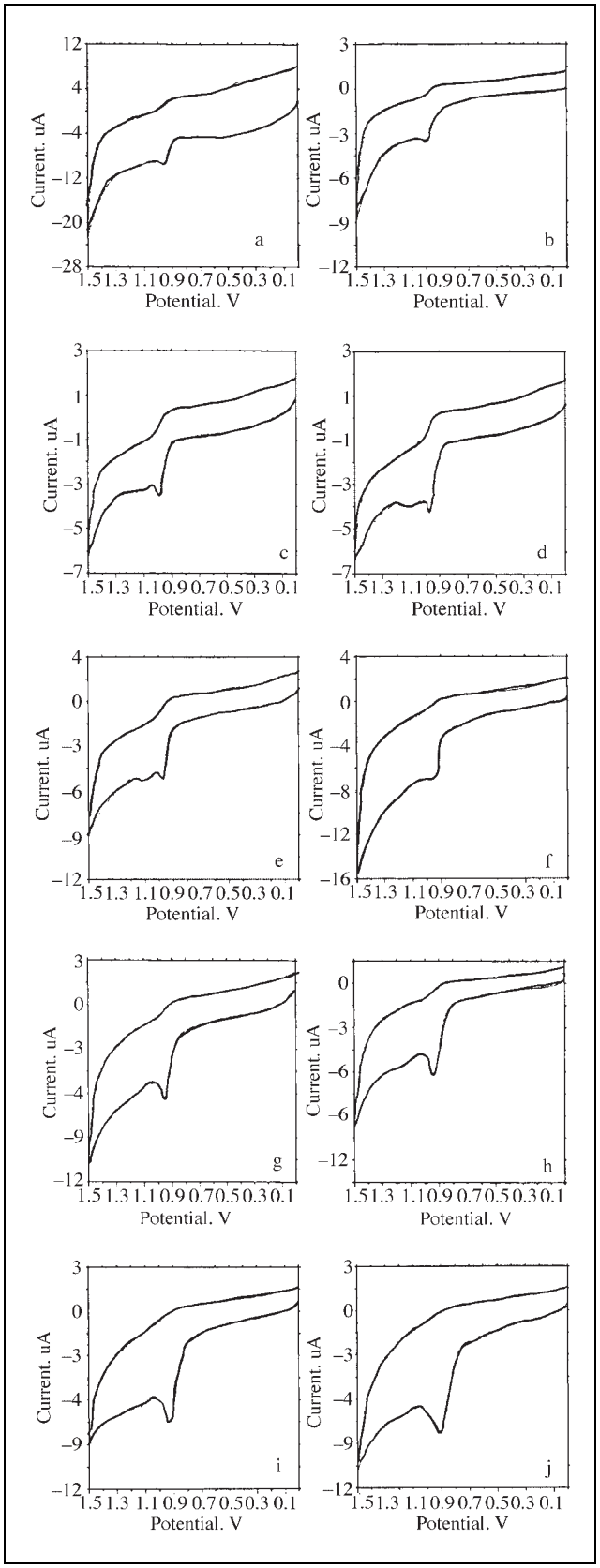

Fig. 1: Cyclic voltammograms of 10^{-4} M paroxetine hydrochloride obtained in; a – 0.5 M H_2SO_4; and Britton-Robinson buffers solution b – 1.83; c – 2.02; d – 3.17; e – 4.00; f – 5.00; g – 6.01; h – 7.24; i – 8.16; j – 9.22, scan rate 100.0 mV s^{-1}

In phosphate buffers of pH = 3.08, 4.04, 5.01, 6.00, 7.25, 8.16 and 11.3 similar peaks were observed but peak currents were smaller in comparison with Britton-Robinson buffers. For analysis, best results were obtained in Britton-Robinson buffer of pH = 7.24 and phosphate buffer of pH = 8.16; these electrolytes were used throughout this study. Figs. 2a and 2b show the relationships between peak current and pH and peak potential and pH for POT, respectively.

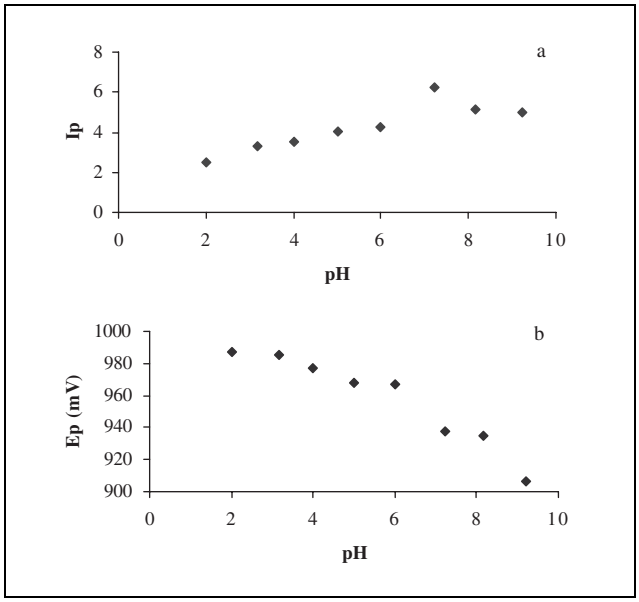

Fig. 2: a: Relationship between peak current and pH. b: Relationship between peak potential and pH

The cyclic voltammograms were recorded in Britton-Robinson buffer of pH = 7.24 containing, 10^{-4} M POT at different scan rates. At lower scan rates a sigmoidal oxidation step was observed. This step changed in peak form and its potential shifted to more positive values as scan rate increased as expected from a nonreversible reaction. The degree of this shift is longer than 100 mV s^{-1} and becomes smaller for higher scan rates. The second peak at about 1.15 V became apparent when scan rate exceeded 75.0 mV s^{-1}; indicating oxidation of an unstable substance.

The relationship existing between peak current and square root of the scan rate was found to be linear: $y = 0.54x + 1.09$ and $y = 0.47x + 0.62$ in Britton-Robinson buffer of pH = 7.24 and phosphate buffers of pH = 8.16, respectively, indicating that the reaction is diffusion controlled. The logaritm of peak current – logarithm of scan rate (log i_p-log v) relationship was also linear: $\log i_p = 0.52 \log v + 0.34$. The slope indicates that the reaction is diffusion controlled and surface reaction effects are not important under the given conditions. From the curves obtained in Britton-Robinson buffer of pH = 7.24, the currents at +1.0 V were read and log i – log C relationship was obtained as $\log i = 2.09 \log C + 0.34$. As can be seen from the slope, the reaction order was found to be 2.

Beginning from a steady-state potential in BR buffer of pH = 7.24 a Tafel plot was obtained with a scan rate of 5 mV s^{-1}. From the slope of the linear part αn was found to be 0.48.

Differential pulse voltammograms were measured with Britton-Robinson buffer solutions of different pH. Best results were obtained in the solution at pH = 7.24 (Fig. 3).

The effect of the POT concentration on peak current at +0.9 V were investigated; a linear relationship was obtained between peak current and concentration. Statistical

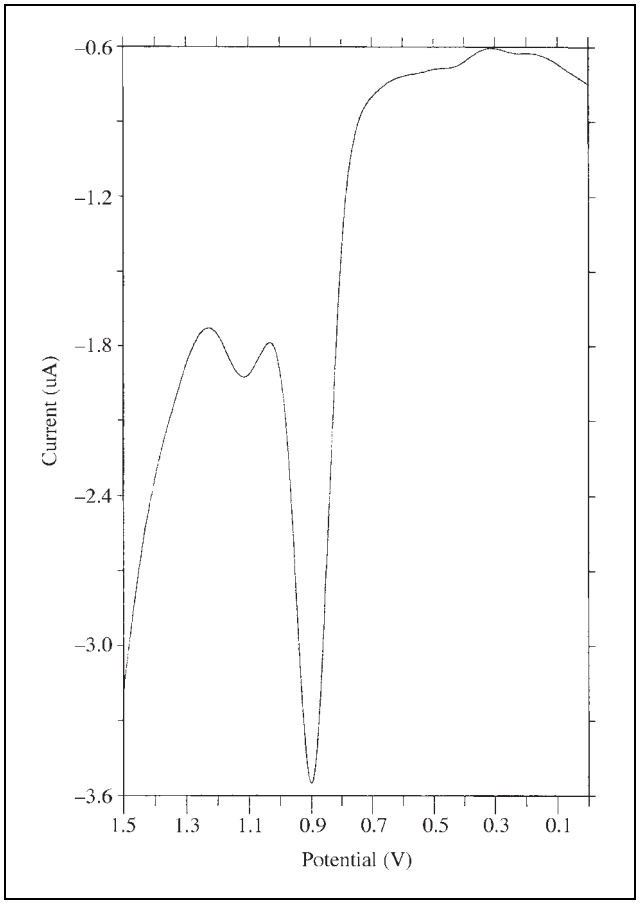

Fig. 3: Differential pulse voltammogram recorded in BR buffers at pH = 7.24 of 10^{-4} M paroxetine hydrochloride, scan rate 20 mV s^{-1}, pulse amplitude 50 mV; sample width 17 ms, pulse width 50 ms, pulse period 200 ms

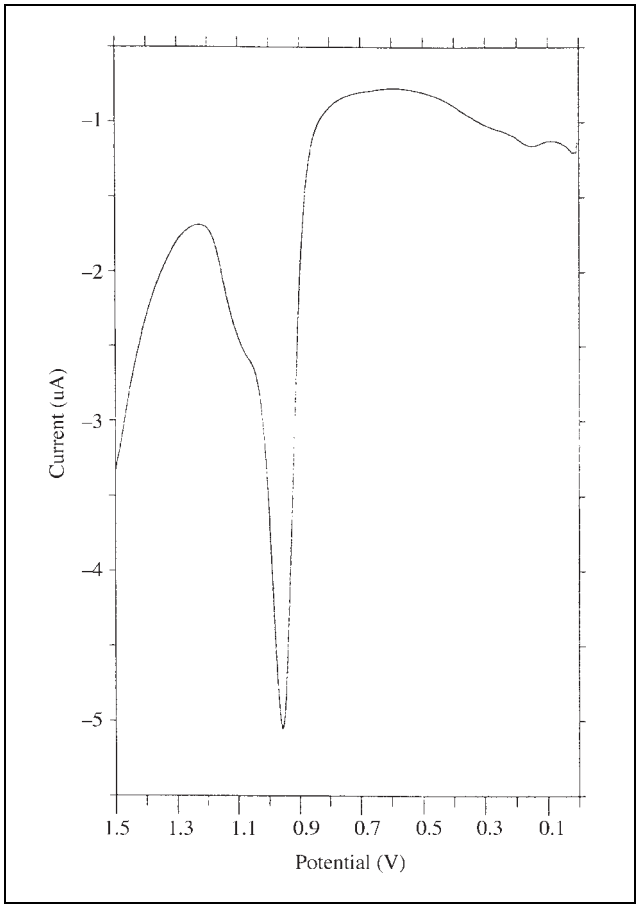

Fig. 4: OSWV curve obtained in BR buffers solutions at pH = 3.17 of 10^{-4} M paroxetine hydrochloride; pulse amplitude, 25 mV; frequency, 15 Hz; potential step 4 mV

analysis of this relationship is given in Table 1. The results showed that quantitative determination of POT could be made by DPV and the optimum conditions were found to be Britton-Robinson buffer pH = 7.24, 9 : 1 v/v methanol : water; 20 mV s^{-1} scan rate, 50 mV pulse amplitude, 17 ms sample width, 50 ms pulse width, 200 ms pulse period.

OSW voltammogram were taken in Britton-Robinson buffer solutions. Best results were obtained in Britton-Robinson buffer of pH = 3.24 (Fig. 4). Optimum OSWV conditions were: pulse amplitude 25 mV; frequency 15 Hz; potential step 4 mV. Under these conditions a linear relationship between peak current and concentration was obtained. The results of the statistical evaluation of these data are shown in Table 1.

The methods were successfully applied to the pharmaceutical dosage forms and human plasma.

2.2. HPLC

The reversed-phase HPLC was developed to provide a specific procedure suitable for the rapid quality control analysis of POT and as referee method for the voltammetric assay.

The mobile phase was investigated after several trials with 0.01 M sodium dihydrogen phosphate : methanol : acetonitrile in various proportions. A mobil phase of 0.01 M sodium dihydrogen phosphate : methanol : acetonitrile (5 : 1 : 4 v/v/v) and flow rate selection was based on peak parameters (height, asymmetry, tailing), baseline drift, run

Table 1: Statistical analysis of calibration graph in the determination of paroxetine hydrochloride by cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (OSWV) and HPLC

Parameters	CV	DPV	OSWV	HPLC
Range (M)	4×10^{-5}–8×10^{-4}	2×10^{-5}–8×10^{-4}	2×10^{-5}–8×10^{-4}	2×10^{-7}–6×10^{-5}
Regression equation (Y)[a]				
Slope (b)	7.23×10^{4}	8.88×10^{4}	2.47×10^{4}	9.32×10^{-2}
Std. dev. on slope (S_b)	3.87×10^{-5}	7.72×10^{-5}	1.81×10^{-4}	1.74×10^{-6}
Intercept (a)	0.66	0.24	0.45	1.56×10^{-2}
Std. dev. on intercept (S_a)	1.19×10^{-7}	8.24×10^{-6}	2.30×10^{-4}	7.41×10^{-6}
Std. error of estimation (S_e)	4.54×10^{-6}	1.98×10^{-5}	9.12×10^{-3}	4.47×10^{-7}
Correlation coefficient (r)	0.9985	0.9996	0.9999	0.9999

[a] Y = a + bC where C is concentration in M and Y in peak area and current units for high performance liquid chromatography and voltammetric methods, respectively
[b] Five replicate samples

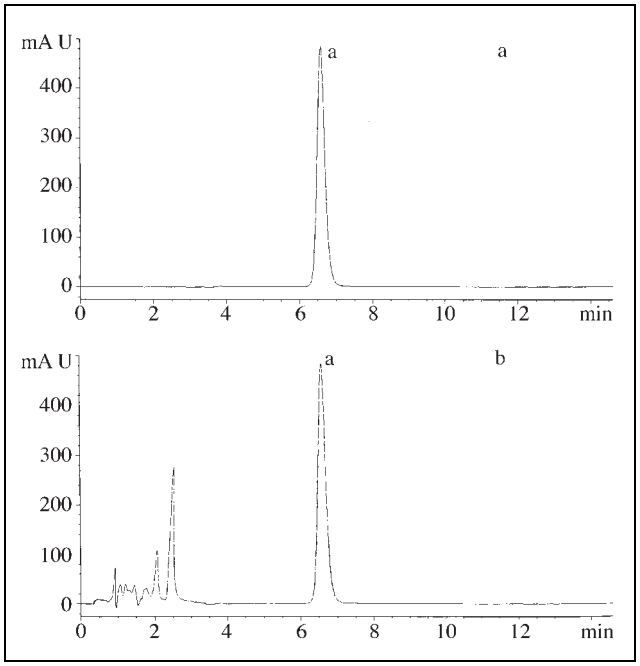

Fig. 5: a: HPLC of a 20 µl injection containing 2×10^{-5} M of paroxetine hydrochlorid b: HPLC of human plasma spiked with 2×10^{-5} M paroxetine hydrochloride (a)

time, ease of preparation of mobile phase. An internal standard was not used as there was no extraction in the estimation of POT in human plasma and in pharmaceutical dosage forms. The system appears to be quite robust. Other ODS columns were tested with minimal effect on the resolution of the analytes. A YMC-ODS-A 132 column is recommended because of its demonstrated ruggedness and reproducibility in this assay. A typical chromatogram for POT with a RP-YMC pack ODS A-132 C_{18} (5 mm, 15 cm × 6.0 mm) column and a flow rate of 1.0 ml/min is shown in Fig. 5a. The optimum wavelength for detection was 237 nm, at which much better detector response for drug was obtained. As shown in the Fig. 5a, a sharp and symmetrical peak was obtained for POT with good baseline and little tailing, thus facilitating the accurate measurement of the peak area. The specificity of the HPLC is illustrated in Fig. 5b, where complete separation of each of POT from biological endogenous components in the human plasma was found. No interfering peaks at the retention time of POT peak were observed in the blank human plasma. The average retention time was 6.60 ± 0.04 min for 10 replicates. In Fig. 5b, it can be seen from the chromatogram that in human plasma, POT is separated from two other peak, without their metabolites.

Under the described HPLC parameters, the respective compounds were base-line separated and were sharply resolved at reasonable retention times. For quantitative analysis, the analytical data for the calibration graphs were obtained with correlation coefficients of 0.9999 for POT (Table 1).

The procedures for the quantitative drug assay were validated with regards to the limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, specificity, and robustness. The LOD and LOQ were calculated from the calibration curves as kSD/b where k = 3 for LOD and 10 for LOQ, where SD is the standard deviation of the intercept and b is the slope of the calibration curve. The values of LOD and LOQ were 4×10^{-6} M (for CV), 2×10^{-6} M (for DPV and OSWV), 6×10^{-8} M for HPLC) and 8×10^{-6} M (for CV) and 6×10^{-6} M (for DPV and OSWV), and 8×10^{-8} M (for HPLC), respectively. Repeatability and recovery were examined by performing five replicate measurements at concentrations of 4×10^{-5}–81^{-4} M POT (for CV), 2×10^{-5}–8×10^{-4} M (for DPV and OSWV), and 2×10^{-7}–6×10^{-5} M (for HPLC), respectively. Mean recoveries of 99.87 ± 0.97 for CV, 99.04 ± 0.47 for DPV, 98.54 ± 1.24 for OSWV and 99.91 ± 0.27 for HPLC were achieved, indicating the usefulness of the application for analysis of pharmaceutical preparations. It was observed that the excipients have no effect on the results.

Specificity of the optimized procedures for assay POT was examined. Recovery tests were performed in order to establish the accuracy and sensitivity of the methods. The results are shown in Table 2. The specificity of the proposed methods is illustrated by the complete separation of each of POT from biological endogenous components in the human plasma. No interfering peaks of POT peak were observed in the blank human plasma.

Robustness was examined by evaluating the influence of small variations of some of the most important procedure variables including pH and concentration potential. The results indicate that none of these variables significantly affect the recovery of POT, providing an indication of the reliability of the proposed procedures for assay of the drug.

The developed method was applied to the analysis of POT in three batches of a commercial formulation. The results presented in Table 3 are in good agreement with the labelled content.

The determination of POT in spiked human plasma samples was carried out at three different concentrations: 2×10^{-5} M, 4×10^{-5} M, and 6×10^{-5} M with DPV, OSWV and HPLC procedures as described above. The recoveries obtained are shown in Table 3. Good recoveries of POT were achieved from biological endogenous components in human plasma.

The HPLC method was chosen as the analytical reference method. The voltammetric methods and first derivative spectrophotometry results were compared with HPLC method by means of student's t-test at 95% confidence level and no significant difference between the methods was found (Table 3).

The three voltammetric methods have the advantage of being rapid, simple, directly applicable to tablet and in-

Table 2: Recoveries of paroxetine hydrochloride from human plasma by voltammetric methods, and HPLC

	Amount (x ± SE, RSD%)[a]			
	CV	DPV	SWV	HPLC
2×10^{-5} M	–	98.7 ± 0.95, 1.49%	97.2 ± 1.08, 1.78%	99.1 ± 0.62, 0.58%
4×10^{-5} M	100.2 ± 1.29, 1.42%	98.4 ± 1.27, 1.92%	97.0 ± 1.90, 1.63%	100.2 ± 0.73, 0.74%
6×10^{-5} M	99.6 ± 1.21, 1.24%	98.7 ± 0.85, 1.54%	99.0 ± 1.45, 1.46%	97.6 ± 0.54, 0.97%

[a] ×, mean; RSD, relative standard deviation; SE, standard error

Table 3: Comparative studies for paroxetine hydrochloride tablets

Drug[a]	CV	DPV Mean [b] ± RSD[c]	OSWV	HPLC
Batch no 1	19.8 ± 0.98	19.9 ± 0.65	19.5 ± 0.74	20.3 ± 0.52
Batch no 2	19.6 ± 1.08	20.5 ± 0.59	19.2 ± 1.24	20.1 ± 0.62
Batch no 3	20.3 ± 1.12	19.8 ± 0.87	20.5 ± 0.92	20.5 ± 1.15
t-test of significant[d]	0.55	0.89	1.02	

[a] Seroxat® film tablets were labeled to contain 20.0 mg paroxetine hydrochloride per tablet
[b] Each value is the mean of ten experiments
[c] RSD = Relative Standard deviation
[d] Values in parentheses are the theoretical values at p = 0.95. Theoretical values at % 95 confidence limits; t = 2.26

expensive compared to HPLC. The HPLC method may be considered more specific than the other methods, but it is also more expensive, requiring sophisticated chromatographic instumentation. All the developed methods may be recommended for routine and quality control analysis of the investigated drug in pharmaceutical dosage forms and in human plasma.

3. Experimental

3.1. Apparatus

Voltammetric measurements were made and curves were obtained with a BAS 100 W/B electrochemical analyser and a HP 1100 laserjet printer. Working and counter electrodes were a BAS MF 2012 glassy carbon disc and a BAS MV 1032 platinum, respectively. A BAS MF 1063 type silver/silver chloride electrode was used as reference. The potantials in the text were given versus silver/silver electrode.
A chromatographic system consisted of a HP 1100 series mode quaternary pump with a HP 1100 series manual injector 20 μL fixed loop, equipped with a diode array and multiple wavelength UV/VIS detectors. The detector was set at 237 nm and peak areas were integrated automatically with the Agilent Chem-Station software programme.

3.2. Materials

Bulk paroxetine hydrochloride was obtained from Novartis Pharm. Ind. Seroxat® film tablets was purchased locally in Turkey. Human plasma samples were obtained from healthy volunteers and stored frozen until assay.
Analytical grade phosphoric acid and HPLC grade methanol, and acetonitrile were purchased from Merck Chem. Ind. All other chemicals were of analytical-reagent grade and were used as received.

3.3. Pharmaceutical preparation

A commercial pharmaceutical preparation (Seroxat® film tablets Novartis Pharm. Ind. TURKEY, containing 20.0 mg of POT) was acquired from a local pharmacy.

3.4. Voltammetric procedure

Stock solutions of concentrations of 2×10^{-5}–8×10^{-4} M POT were prepared in methanol and stored in dark bottles at +4 °C. The working solutions under voltammetric investigations were prepared by dilution of the stock solution and contained 9 : 1 (v/v) methanol:water. The POT solution is stable. Britton-Robinson (BR) buffer (0.04 M) in the pH range 1.5–10.0 was used as a supporting electrolyte when studying the influence of pH. The pH was adjusted to the desired value by adding the required volume of a 5.0 M NaOH solution. Phosphate buffer (0.05 M) in the pH range 3.0–10.0 was used as supporting electrolyte when studying the influence of pH. The pH was adjusted to the desired value by adding the required volume of a phosphoric acid solution.

3.5. Pretreatment of the working electrode

Before each experiment the surface of the glassy carbon (GC) electrode was polished with alumina ($\phi = 0.01$ μm) on a polishing pad and then carefully washed with bidistilled water and dried on a filter paper.

3.6. Chromatographic conditions

HPLC was conducted with a reversed phase technique. The mobile phases used were 0.01 M sodium dihydrogen phosphate : methanol : acetonitrile (5 : 1 : 4 v/v/v), and the analytical column was a RP-YMC pack ODS A-132 C_{18} (5 m, 15 cm × 6.0 mm) column. All analysis were done with an isocratic system at a flow rate of 1.0 mL min^{-1} at room temperature. A diode array detector was fixed at 237 nm. All solvents were filtered through a 0.45 μm milipore filter before use and degassed in an ultrasonic bath.

3.7. Chromatographic procedure

Stock solutions of concentration 10^{-4} M POT were prepared in methanol and stored in dark bottles at +4 °C. Working solutions of 2×10^{-7}–6×10^{-5} M were prepared by the appropriate dilutions of the stock standard with mobile phase. The working solutions were prepared freshly every day.

3.8. Analysis of pharmaceutical dosage forms

Ten Seroxat® film tablets (Novartis Pharm. Ind. amount declared of POT per tablet 20.0 mg) were triturated in an agate mortar, pounded and finally the correct amount of powder was dissolved in 9 : 1 (v/v) methanol : water by stirring for 30 min. The excipient was separated by filtration and the residue washed three times with same solvent. The solution was transferred quantitatively into a calibrated flask and diluted to a final volume of 500.0 ml with same solvent thus a stock of 10^{-4} M was prepared. All the test solutions were obtained by diluting this stock solution with the selected supporting electrolyte. Voltammograms were recorded following the voltammetric procedure. The HPLC determination of POT was made by adding an aliquot of the above mentioned solution to the mobile phase and then these solutions were filtered through 0.45 μm membrane filters. Triplicate 20 μL injections were made for each solution.

3.9. Recovery studies

To make an additional check on the accuracy of the developed assay methods and to study the interference of formulation additives, analytical recovery experiments were performed by adding a known amount of pure drug to the preanalysed samples of commercial dosage forms. The percent analytical recovery values calculated by comparing concentration obtained from the spiked samples with actual added concentrations are also listed.

3.10. Spiked human plasma samples

First, 0.5 ml of human plasma was mixed with acetonitrile (1.0 ml) and with standard solution of POT from, to give drug concentrations of $4 \times 10^{-5} - 8 \times 10^{-4}$ M for voltammetric techniques and 2×10^{-7}–6×10^{-5} M for HPLC analysis. Addition of acetonitrile prevents POT from binding to proteins and coagulating plasma proteins. The mixtures were vortexed for 10 min. After deproteinization and centrifugation of sample for 15 min at 6000 rpm the supernatant (1 ml) was separated. Appropriate volumes of this solution were added to selected supporting electrolyte and the voltammograms were recorded following.
HPLC analysis of the prepared solutions was performed on 20 μL samples. No anticoagulant was used in these proposed method.

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